A Thesis
For the Degree of Master of Veterinary Medicine

Increased expression of activin A in the spinal cords of rats with clip compression injuries

Department of Veterinary Medicine

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

Jinwoo Jeong

2012.2.

Increased expression of activin A in the spinal cords of rats with clip compression injuries

Jinwoo Jeong

(Supervised by Professor Taekyun Shin)

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Veterinary Medicine

2011. 12

This thesis has been examined and approved.

_____

Thesis director, Ki-Bum Sim,

Prof. of Dept. of Neurosurgery

_____

Hwang-Lyong Kim, PhD, DVM

_____

Taekyun Shin, Prof. of Dept. of Veterinary Medicine

2012. 2

Department of Veterinary Medicine

GRADUATE SCHOOL

CONTENTS

¥°. Abstract

Activin A, a member of transforming growth factor β superfamily, plays roles in neuroprotection and immunomodulation. The expression of activin A was studied in the inflammatory lesions of rats with spinal cord injury (SCI) by Western blotting and immunohistochemistry. Western blotting showed that levels of activin A in core lesions in rats with SCI were significantly increased at day 1 (p<0.05) and day 4 post-injury (p<0.01) compared to sham control spinal cords, and were significantly lower at day 7 post-injury compared to day 4 post-injury (p<0.05). Immunohistochemical analysis revealed constitutive expression of activin A in vessels and neurons in the spinal cords of sham control rats. After SCI, activin A was also detected in inflammatory cells in the core lesion, particularly in isolectin B4-positive macrophages and glial cells. Collectively, our results suggest that a transient increase in activin A levels after clip compression injury may contribute to the modulation of inflammation during SCI.

Keywords : activin A; spinal cord injury; macrophage; astrocyte

¥±. Materials and methods

Ⅱ.1. Animals

Sprague-Dawley rats (200–250 g, female) (OrientBio Inc., Kyunggido, Korea) were used. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at Jeju National University.

Ⅱ.2. Antibodies and antisera

Mouse monoclonal anti-activin A (clone number 69403, IgG1; R&D systems Inc., Minneapolis, MN, USA) and mouse monoclonal anti-β-actin (Sigma-Aldrich, St. Louis, MO, USA) antibodies were used. Cell phenotypes were identified using the following antibodies: rabbit polyclonal anti-GFAP (Dako, Copenhagen, Denmark) for astrocytes; biotinylated IB4 (Sigma-Aldrich) for vascular endothelial cells and macrophages; and mouse monoclonal anti-CNPase (Millipore, Billerica, MA, USA) for oligodendrocytes.

Ⅱ.3. Spinal cord injury

The clip compression injury procedure was performed using previously published methods (Jung et al., 2003; Kim et al., 2003, Moon et al., 2004) with modifications. The animals were anesthetized and subjected to laminectomy at T9/T10. Immediately following laminectomy, the spinal cord was compressed with a vascular clip (Stoelting, Wood Dale, IL, USA), applied vertically to the exposed spinal cord at an occlusion pressure of 15–20 g for 1 min. After compression, the muscles and skin layers were closed. A total of 20 rats were used in this study. In sham-operated control rats, only the laminectomy procedure was performed. After injury to the spinal cord, and at the time tissues were obtained, the animals lacked all hind-limb locomotion. The animals were sacrificed at day 0 (sham-operated controls, n=5), and at days 1 (n=5), 4 (n=5), and 7 (n=5) PI. Spinal cord tissues from the surgical sites were harvested and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) for histological examination and Western blot analysis, respectively.

Ⅱ.4. Western blot analysis

Western blot analysis was performed as described previously (Jung et al., 2003; Moon et al., 2004). Briefly, spinal cords were dissected free, minced, homogenized, and lysed in a buffer containing 40 mM Tris-HCl (pH 7.4), 120 mM NaCl, and 0.1% Nonidet P-40 (polyoxyethylene [9] p-t-octyl phenol) and supplemented with the protease inhibitors leupeptin (0.5 μg/ml), phenylmethanesulfonyl fluoride (1 mM), and aprotinin (5 μg/ml). Equal amounts of protein (60 μg/20 μl) were loaded to each lane of a polyacrylamide gel and separated under denaturing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, the proteins were electrotransferred onto nitrocellulose transfer membranes (Schleicher and Schuell, Keene, NH, USA). Activin A was detected using mouse monoclonal anti-activin A (R&D systems Inc.). Immunoreactive bands were visualized by chemiluminescence using a horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Vector, Burlingame, CA, USA) and enhanced chemiluminescence (ECL; Amersham, Arlington Heights, IL, USA). The membranes were reprobed with a monoclonal antibody specific for β-actin (Sigma-Aldrich).

Ⅱ.5. Immunohistochemistry

Spinal cords were fixed for 24 h and processed for paraffin embedding. Then, 5-μm-thick sections of paraffin-embedded spinal cords were deparaffinized and allowed to react with mouse anti-activin A antibody (dilution approximately 1:100–1:200). To identify individual cell types in the spinal cords, the following primary antibodies were applied: anti-GFAP (dilution 1:400) for astrocytes, anti-CNPase (1:1,000) for oligodendrocytes, and biotinylated IB4 (1:50) for macrophages and vessels. Immunoreactivity was visualized with

an avidin-biotin peroxidase reaction (Vector Elite, Vector Labs). The peroxidase reaction was developed using a diaminobenzidine substrate kit (Vector). To describe the cell phenotypes of both activin A and oligodendrocytes or vessels, serial mirror sections were subjected to single immunostaining as described above, as both antibodies originated from the same mouse. Staining intensity was evaluated under an Olympus microscope. Three different sections from three animals per group were examined by three blinded observers. By semi-quantitative analysis, samples were defined as being negative (–) or containing <20 positive cells (+), 20–50 positive cells (++), or >50 positive cells (+++). The intensity of activin A staining in endothelial cells of vessels was classified qualitatively (at ×20 magnification) as weak (+), moderate (++), or intense (+++) by three blinded observers.

Ⅱ.6. Double immunofluorescence

For double-labeling of two antigens, paraffin sections were sequentially treated with 10% normal goat serum for 1 h, mouse monoclonal anti-activin A (dilution 1:100) at 4°C overnight, biotinylated anti-mouse IgG (1:200, Vector Labs), and tetramethylrhodamine isothiocyanate-labeled streptavidin (1:500, Zymed, San Francisco, CA, USA) for 1 h at room temperature (RT). They were next washed and incubated for 1 h at RT with either rabbit polyclonal anti-GFAP (1:400, Sigma-Aldrich) or biotinylated IB4 (1:50, Sigma-Aldrich). After washing, the sections were incubated with either fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1:50, Sigma-Aldrich) or fluorescein isothiocyanate-labeled streptavidin (1:500, Zymed) for 1 h at RT. To minimize lipofuscin autofluorescence, the sections were next washed in PBS for 1 h at RT three times, dipped briefly in distilled water, treated with

10 mM copper sulfate in 50 mM ammonium acetate buffer (pH 5.0) for 20 min, briefly dipped in distilled water again, and returned to PBS. Stained sections were examined under a BX-51 fluorescence microscope (Olympus, Tokyo, Japan). Captured images were merged using Adobe Photoshop 7.0 software (Adobe Systems, San Jose, CA, USA) to allow assessment of co-localization.

¥². Results

Ⅲ.1. Behavioral changes and histopathological findings following SCI

After clip compression injury in rat spinal cords, all rats showed complete hindlimb paralysis. Although no hindlimb paralysis was observed in sham-operated control rats, all SCI rats with Basso, Beattie, and Bresnahan (BBB) locomotor rating scale (Basso et al., 1995) scores of '0' (data not shown) were subjected to histological examination. In the spinal cords of sham-operated control rats, no pathological lesions were found in longitudinal (Fig. 1A) or cross-sectional (Fig. 1C) sections. At day 1 post-injury (PI), there was hemorrhage, edema, and neural loss in the core spinal cord lesions (Fig. 1D). At day 4 PI, in longitudinal section analysis, in areas extending approximately 5 mm from the core lesions in the cranial and caudal directions, edema, hemorrhage, and vacuoles were found (Fig. 1B). Massive infiltration of macrophages was found at injury sites in the cross-sections (Fig. 1E). At day 7 PI, histological lesions were similar to those at day 4 (Fig. 1F). All pathological findings in this study were largely similar to those of our previous studies (Jung et al., 2003; Kim et al., 2003; Moon et al., 2004a; 2004b; Shin , 2007; Song et al., 2009).

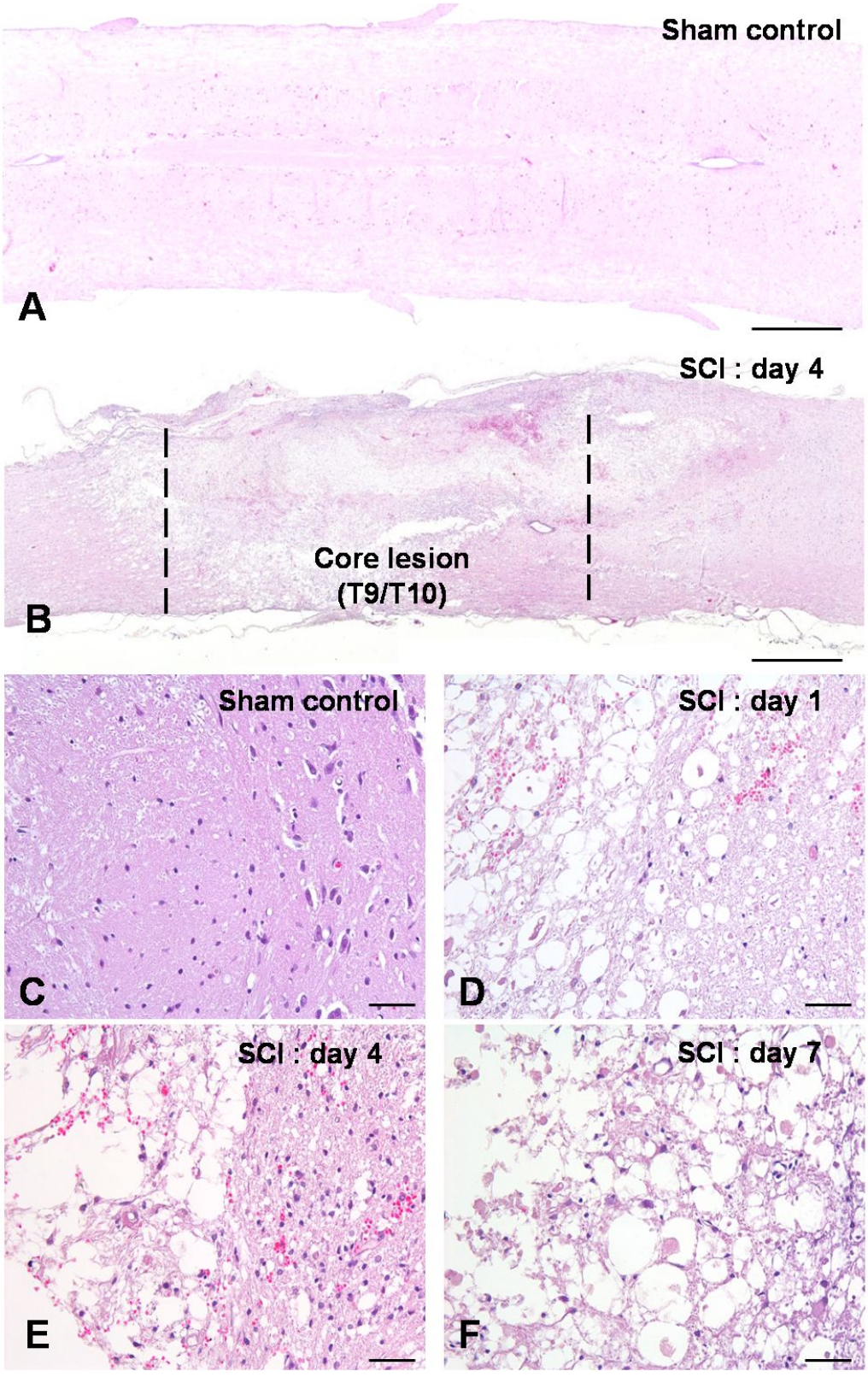
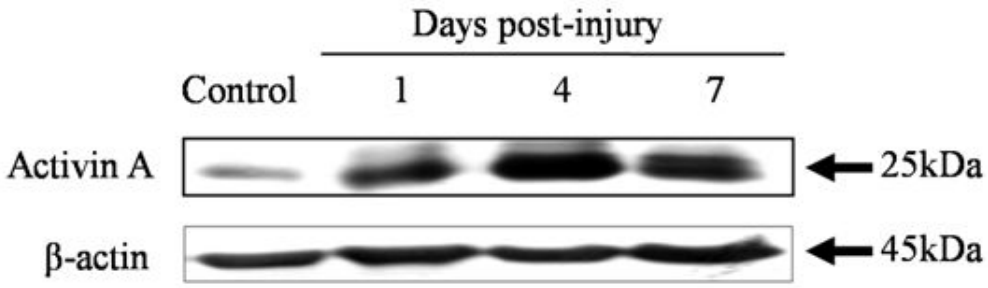


Figure 1. Histopathological findings in spinal cord injury (SCI). Histological findings in the spinal cords of sham control rats (A and C) and rats with SCI at days 1 (D), 4 (B and E), and 7 PI (F). (A and B) Longitudinal sections of spinal cord lesions approximately 5 mm cranial and caudal to the core lesion. (C-F) Cross-sections of core lesions of spinal cords from sham-operated control rats (C) and injured rats (D-F). Dotted line: clip injury boundary. Hematoxylin-eosin staining. Scale bars: (A and B), 1,000 μm; (C-F), 50 μm.

Ⅲ.2. Western blot analysis of activin A

Western blot analysis was used to examine changes in activin A protein levels in the spinal cord after compression injury (Fig. 2). Activin A was quite weakly detected in spinal cords from sham-operated control rats, and its expression was arbitrarily given a value of 1. Levels were slightly increased in the spinal cord at day 1 PI (relative optimal density [OD], 2.93-fold ± 0.54, n=3, p<0.05), peaked at day 4 PI (relative OD, 4.10-fold ± 0.85, n=3, p<0.01), and were slightly lower at day 7 PI (relative OD, 2.63-fold ± 0.04, n=3, p<0.05).

A



B

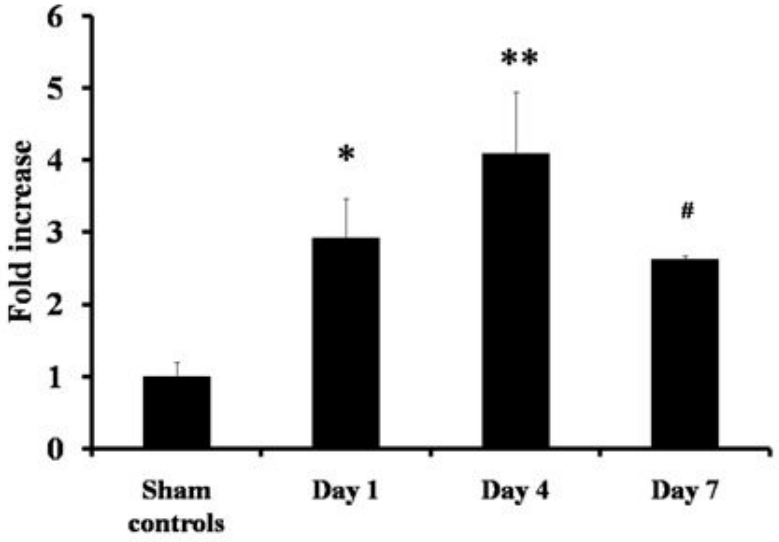


Figure 2. Western blot analysis of activin A expression in the spinal cords of rats with SCI. (A) Representative images of Western blot analysis of activin A and β-actin in the spinal cords of sham control rats and rats with SCI at days 1, 4, and 7 PI. Arrows indicate activin A (approximate molecular weight 25 kDa) and β-actin (45 kDa) protein bands. Lane 1, sham control (Sham); lane 2, SCI day 1 PI; lane 3, SCI day 4 PI; lane 4, SCI day 7 PI. (B) Bar graphs of semi-quantitative densitometric data (mean±SE, n=3 per group). Relative activin A expression in each sample was calculated after normalization to the β-actin signal. Sham controls were arbitrarily given a value of 1. *p<0.05, **p<0.01 vs. sham controls; $^{\#}p$<0.05 vs. day 4 PI.

Ⅲ.3. Immunohistochemical localization of Activin A

In the spinal cords of sham-operated rats, activin A immunostaining in the gray matter was weakly detected at low magnification (Fig. 3A). At high magnification, it was also weakly detected in some vessels and neurons (Fig. 3B, arrows). At day 1 PI, activin A immunoreactivity was diffuse in disorganized spinal cords, as assessed by visualization at low magnification (Fig. 3C); positive neurons and glial cells were detected at a higher magnification (Fig. 3D). The immunoreactivity was stronger at day 4 PI than at day 1 PI, as assessed at low magnification (Fig. 3E). At day 4 PI, it was detected in some glial cells (Fig. 3F, arrowheads), as well as some large cells with cytoplasmic granules, including phagocytes (Fig. 3F, arrows) and neurons (Fig. 3F). At day 7 PI, the immunoreactivity, as detected at low magnification, was weaker than at day 4 PI (Fig. 3G). Activin A-positive cell patterns at day 7 PI were similar to that at day 4 PI (Fig. 3H).

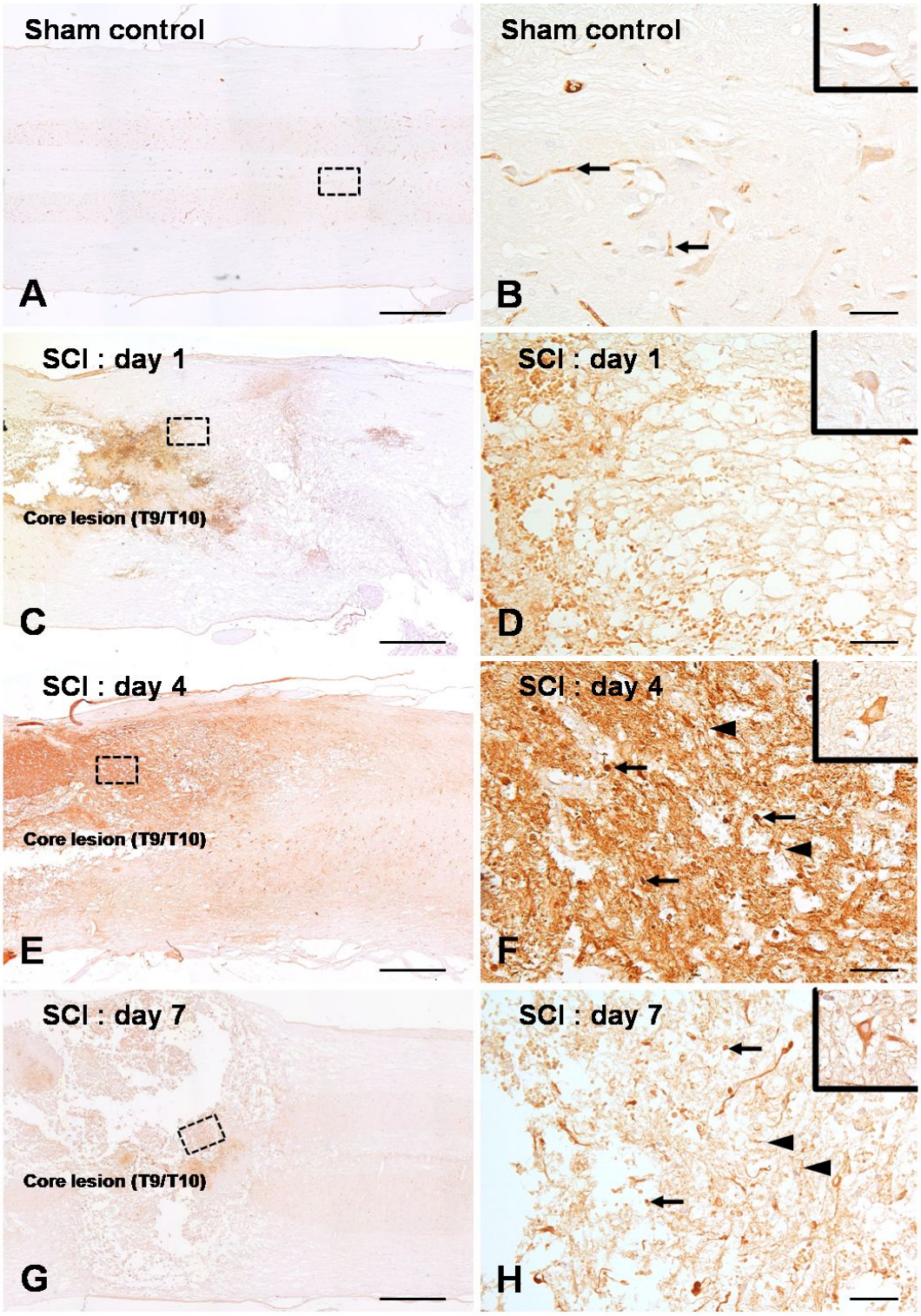


Figure 3. Immunohistochemical staining of activin A in spinal cords from sham control rats (A and B) and from SCI rats at days 1 (C and D), 4 (E and F), and 7 PI (G and H). (B, D, F, and H) Higher-magnification images of (A, C, F, and H), respectively. In spinal cords from sham control rats, activin A was detected in some vessels (B, arrows) and neurons (B, inset). In the injured spinal cords, diffuse activin A immunostaining was detected in core and peripheral lesions at day 1 PI (C and D). At day 4 PI (E and F), large phagocytic cells (F, arrows) with cytoplasmic granules, glial cells (F, arrowheads), and the cytoplasm of neurons (F, inset) showed increased immunoreactivity to activin A. At day 7 PI (G and H), fewer activin A-positive large cells (H, arrows) and glial cells (H, arrowheads) were present in the lesions. Sections were counterstained with hematoxylin. Scale bars: (A, C, E, and G), 1,000 μm; (B, D, F, and H), 50 μm.

Ⅲ.4. Cell phenotype of activin A immunoreactivity in SCI lesions

To confirm the phenotypes of cells that express activin A in SCI rats, spinal cord sections were subjected to double immunofluorescence staining for anti-activin A and either anti-isolectin B4 (IB4) or glial fibrillary acidic protein (GFAP). The majority of activin A-positive cells were also positive for IB4 (Fig. 4C) or GFAP (Fig. 4F). These results suggest that the round cells and glial cells may be macrophages and astrocytes, respectively. Activin A was detected in oligodendrocytes and vessels in mirror sections immunostained with anti-2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) or IB4 and anti-activin A, respectively, indicating that activin A (Fig. 5B and D) co-localized with CNPase (Fig. 5A) and IB4 (Fig. 5C), confirming that oligodendrocytes and vessels express activin A. The results of immunohistochemical analysis of activin A expression in SCI rats are summarized in Table 1.

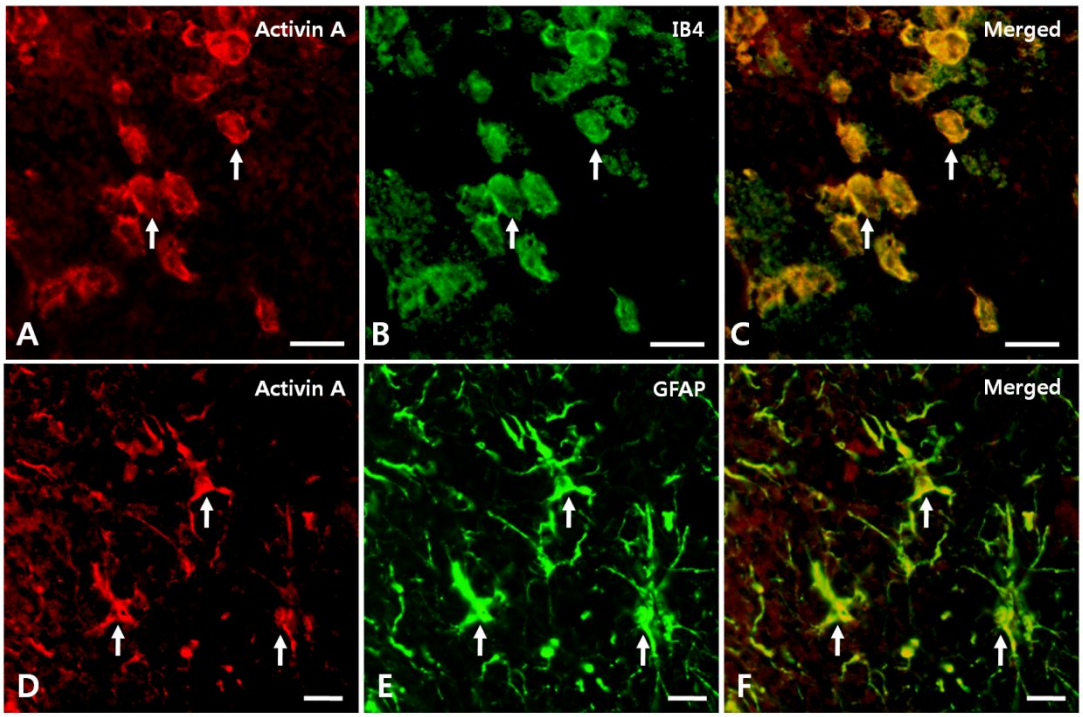


Figure 4. Double immunofluorescence staining of SCI sections at day 4 PI was performed to detect activin A (red) and IB4 (green) (A-C) or activin A (red) and GFAP (green) (D-F). Arrows indicate activin A-expressing IB4-positive macrophages and GFAP-positive astrocytes. Scale bars: 20 μm.

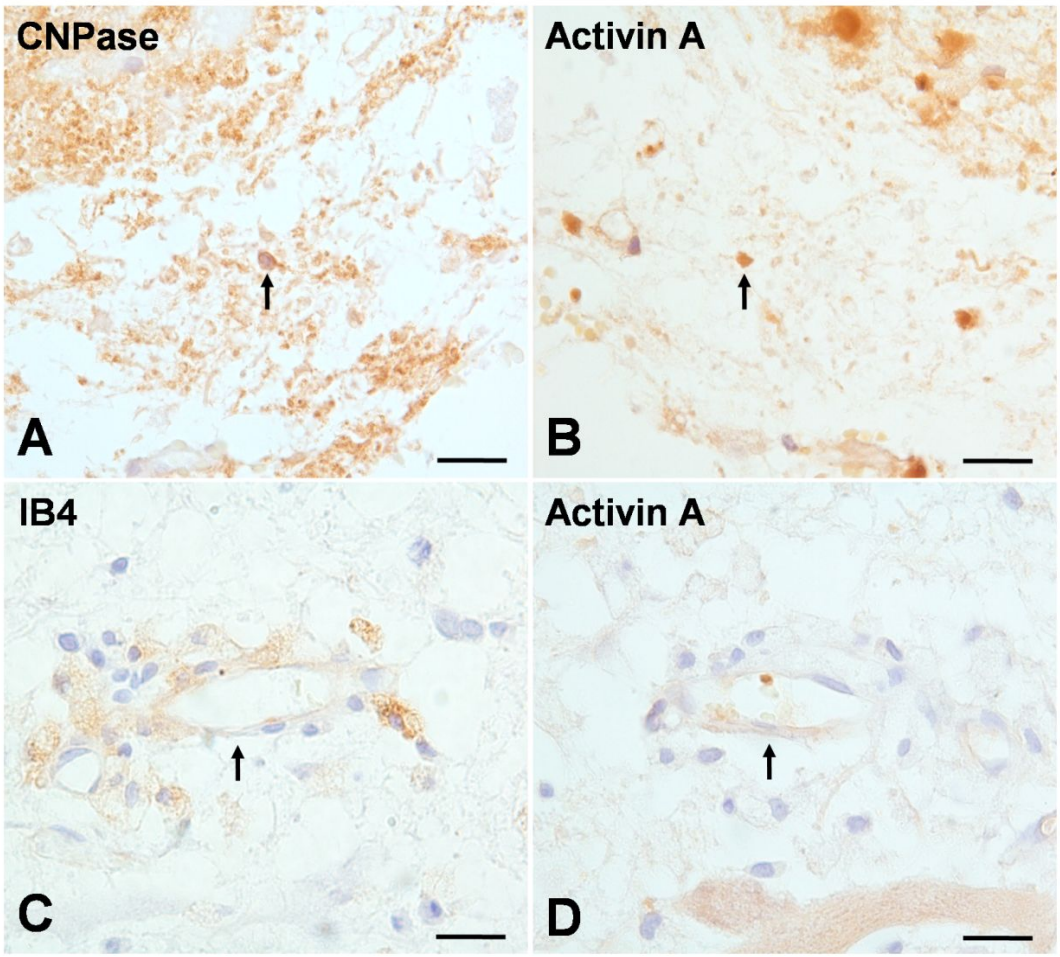


Figure 5. Immunohistochemical localization of CNPase, activin A, and IB4 at day 4 PI in mirror sections of spinal cords. CNPase (A; arrow) and IB4 (C; arrow) colocalized with activin A (B and D; arrows) in mirror sections of spinal cords. The sections shown in (A and B) and (C and D) are serial mirror sections. Sections were counterstained with hematoxylin. Scale bars: 20 μm

Table 1. Activin A immunoreactivity in the spinal cords of control and spinal cord injury (SCI) rats.[a]

Cell type	Sham controls	SCI[b]		
		Day 1 PI	Day 4 PI	Day 7 PI
Macrophages	ND[c]	+[d]	+++	++
Astrocytes	-	+	++	++
Oligodendrocytes	-	+	+	+
Neurons	+[e]	+	++	++
Vascular endothelial cells	+[e]	+	+	+

[a]Three different sections from three animals per group were examined by three blinded observers.

[b]Spinal cords were obtained from rats at day 1, 4, and 7 post-injury (PI).

[c]ND, macrophages not detected in the spinal cords

[d]Activin A-immunoreactive cells in the spinal cords of rats in each group were scored (at ×20 magnification) as negative (–), <20 positive cells (+), 20–50 positive cells (++), and >50 positive cells (+++).

[e]Intensity of activin A staining in neurons and endothelial cells of vessels was classified as weak (+), moderate (++), or intense (+++) by three blinded observers.

¥³. Discussion

This is the first report of the increased expression of activin A in SCI lesions. We examined the activin A response during acute severe SCI induced using vascular clips. Activin A was constitutively expressed in the spinal cords of sham-operated control rats. Levels increased at days 1 and 4 PI, and declined between day 4 and day 7 PI. Our data suggest that overexpression of activin A after SCI plays an important role in inflammation in the central nervous system, such as that associated with SCI.

Immunohistochemical analysis of spinal cords from sham control rats detected activin A in vessels and neurons. We believe that major sources of activin A were the cerebrospinal fluid and the plasma, because it was diffusely detected in the parenchyma at day 1 PI, as previously reported (Michel et al., 2003) in rabbits with bacterial meningitis associated with activation of the microglia (Michel et al., 2003). At days 4 and 7 PI, the major sources of activin A were astrocytes, macrophages, and neurons. However, expression of activin A was decreased at day 7 PI.

In the CNS, activin A is produced by neurons. Its production is elevated following neuronal damage, suggesting that it may participate in protective mechanisms (Florio et al., 2007). In an animal model of excitotoxic injury, activin A produced neuroprotective effects in combination with basic fibroblast growth factor (bFGF) (Tretter et al., 2000). Activin A released from astrocytes inhibits the gliotic response (Abdipranoto-Cowley et al., 2009). In addition, activin A signaling stimulates neurogenesis after neurodegeneration by inhibiting inflammatory and gliotic mechanisms (Abdipranoto-Cowley et al., 2009). Activin expression

has been detected in many immune cells, including macrophages (Ebert et al., 2007). Secreted activin A negatively regulates the production of cytokines and chemokines (Robson et al., 2008). Activin A in macrophages may thus prevent the uncontrolled release of cytokines/chemokines. In microglia, activin β_A mRNA was detected after hypoxic-ischemic injury (Michel et al., 2003).

In a previous study, treatment with the activin A antagonist follistatin showed that neurogenesis does not proceed after neurodegeneration in a kainic acid-induced animal model (Abdipranoto-Cowley et al., 2009). We believe that injection of follistatin in an SCI model reduces the activation of activin A, and thus delays neurogenesis and recovery from paralysis in SCI.

In conclusion, activin A was mainly expressed in macrophages, astrocytes, and neurons in SCI lesions, and possibly has beneficial neuroprotective and anti-inflammatory roles. We propose that increased activin A levels in CNS lesions help to modulate neurodegeneration. However, defining the precise role of activin A in SCI requires further study.

References

Abdipranoto-Cowley, A., Park, J.S., Croucher, D., Daniel, J., Henshall, S., Galbraith, S., Mervin, K., Vissel, B., 2009. Activin A is essential for neurogenesis following neurodegeneration. Stem Cells 27, 1330-1346.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12, 1-21.

Brosh, N., Sternberg, D., Honigwachs-Sha'anani, J., Lee, B.C., Shav-Tal, Y., Tzehoval, E., Shulman, L.M., Toledo, J., Hacham, Y., Carmi, P., Jiang, W., Sasse, J., Horn, F., Burstein, Y., Zipori, D., 1995. The plasmacytoma growth inhibitor restrictin-P is an antagonist of interleukin 6 and interleukin 11. Identification as a stroma-derived activin A. J. Biol. Chem. 270, 29594-29600.

de Kretser, D.M., Loveland, K.L., Meehan, T., O'Bryan, M.K., Phillips, DJ., Wreford, NG., 2001. Inhibins, activins and follistatin: actions on the testis. Mol. Cell. Endocrinol. 180, 87-92.

Ebert, S., Zeretzke, M., Nau, R., Michel, U., 2007. Microglial cells and peritoneal macrophages release activin A upon stimulation with Toll-like receptor agonists. Neurosci. Lett. 413, 241-244.

Florio, P., Gazzolo, D., Luisi, S., Petraglia, F., 2007. Activin A in brain injury. Adv. Clin. Chem. 43, 117-130.

Funaba, M., Ikeda, T., Ogawa, K., Murakami, M., Abe, M., 2003. Role of activin A in

murine mast cells: modulation of cell growth, differentiation, and migration. J. Leukoc. Biol. 73, 793-801.

Jones, K.L., Brauman, J.N., Groome, N.P., de Kretser, D.M., Phillips, D.J., 2000. Activin A release into the circulation is an early event in systemic inflammation and precedes the release of follistatin. Endocrinology 141, 1905-1908.

Jones, K.L., de Kretser, D.M., Patella, S., Phillips, D.J., 2004. Activin A and follistatin in systemic inflammation. Mol. Cell. Endocrinol. 225, 119-125.

Jung, K., Min, D. S., Sim, K. B., Ahn, M., Kim, H., Cheong, J. and Shin, T., 2003. Upregulation of phospholipase D1 in the spinal cords of rats with clip compression injury. Neurosci. Lett. 336, 126-130.

Kim, D. H., Heo, S. D., Ahn, M., Sim, K. B. and Shin, T., 2003. Activation of embryonic intermediate filaments contributes to glial scar formation after spinal cord injury in rats. J. Vet. Sci. 4, 109-112.

Michel, U., Gerber, J., E O'Connor, A., Bunkowski, S., Brück, W., Nau, R., Phillips, D.J., 2003. Increased activin levels in cerebrospinal fluid of rabbits with bacterial meningitis are associated with activation of microglia. J. Neurochem. 86, 238-245.

Moon, C., Heo, S., Ahn, M., Kim, H., Shin, M., Sim. K. B., Kim, H. M. and Shin, T., 2004a. Immunohistochemical study of osteopontin in the spinal cords of rats with clip compression injury. J. Vet. Med. Sci. 66, 1307-1310.

Moon, C., Heo, S., Sim, K.B., Shin, T., 2004b. Upregulation of CD44 expression in the spinal cords of rats with clip compression injury. Neurosci. Lett. 367, 133-136.

Ogawa, K., Funaba, M., Chen, Y., Tsujimoto, M., 2006. Activin A functions as a Th2

cytokine in the promotion of the alternative activation of macrophages. J. Immunol. 177, 6787-6794.

Phillips, D.J., de Kretser, D.M., Hedger, M.P., 2009. Activin and related proteins in inflammation: not just interested bystanders. Cytokine Growth Factor Rev. 20, 153-164.

Prewitt, C. M., Niesman, I. R., Kane, C. J. and Houle, J. D. 1997. Activated macrophage /microglial cells can promote the regeneration of sensory axons into the injured spinal cord. Exp. Neurol. 148, 433-443.

Robson, N.C., Phillips, D.J., McAlpine, T., Shin, A., Svobodova, S., Toy, T., Pillay, V., Kirkpatrick, N., Zanker, D., Wilson, K., Helling, I., Wei, H., Chen, W., Cebon, J., Maraskovsky, E., 2008. Activin-A: a novel dendritic cell-derived cytokine that potently attenuates CD40 ligand-specific cytokine and chemokine production. Blood 111, 2733-2743.

Satoh, M., Sugino, H., Yoshida, T., 2000. Activin promotes astrocytic differentiation of a multipotent neural stem cell line and an astrocyte progenitor cell line from murine central nervous system. Neurosci. Lett. 284, 143-146.

Semitekolou, M., Alissafi, T., Aqqelakopoulou, M., Kourepini, E., Kariyawasam, H. H., Kay, A. B., Robinson, D. S., Lloyd, C. M., Panoutsakopoulou, V., Xanthou, G., 2009. Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. J. Exp. Med. 206, 1769-1785.

Shin. T., 2007. Increases in the phosphorylated form of caveolin-1 in the spinal cord of rats with clip compression injury. Brain Res. 1141, 228-234.

Song, M.S., Seo, H.S., Yang, M., Kim, J.S., Kim, S.H., Kim, J.C., Wang, H., Sim, K.B., Kim, H., Shin, T., Moon C. 2009. Activation of Ca2+/calmodulin-dependent protein kinase II alpha in the spinal cords of rats with clip compression injury. Brain Res. 1271, 114-120.

Tretter, T.P., Herte, M., Munz, B., ten Bruggencate, G., Werner, S., Alzheimer, C., 2000. Induction of activin A is essential for the neuroprotective action of basic fibroblast growth factor in vivo. Nat. Med. 6, 812-815.

Wilms, H., Schwark, T., Brandenburg, L.O., Sievers, J., Dengler, R., Deuschl, G., Lucius, R., 2010. Regulation of activin A synthesis in microglial cells: Pathophysiological implications for bacterial meningitis. J. Neurosci. Res. 88, 16-23.

Ying, S.Y., 1987. Inhibins and activins: chemical properties and biological activity. Proc. Soc. Exp. Biol. Med. 186, 253-264.

Ying, S.Y., Zhang, Z., Haun, C.K., 1996. Localization of activin and activin receptors in rat spinal motoneurons. J. Biomed. Sci. 3, 187-190.

Zhou, J., Tai, G., Liu, H., Ge, J., Feng, Y., Chen, F., Yu, F., Liu, Z., 2009. Activin A down-regulates the phagocytosis of lipopolysaccharide-activated mouse peritoneal macrophages in vitro and in vivo. Cell. Immunol. 255, 69-75.

초 록

클립을 이용한 랫트의 척수손상 모델에서 Activin A 의 발현 증가

(지도교수 : 신 태 균)

정 진 우

제주대학교 일반대학원 수의학과

Activin A 는 transforming growth factor β 의 아과의 한 종류로 알려진 연구결과에 따르면 신경보호, 면역조절들의 기능을 갖는 것으로 알려져 있다. 본 연구에서는 클립을 이용한 랫트의 척수손상 모델에서 activin A의 발현을 확인하였다. SCI(spinal cord injury)는 랫트의 척수손상 모델 중 하나로 신경조직 손상, 염증반응, 기능 손실 등의 특징을 나타낸다. SCI 에서 activin A 의 발현을 알아보고자 웨스턴블럿, 면역조직화학법, 면역형광염색법을 사용하여 본 연구를 진행하였다. 웨스턴블럿 결과 core lesion에서 activin A는 정상대조군에 비해 실험군에서 의미있는 증가를 나타내었고 SCI 4일째에 비해 7일째에 감

소하는 경향을 보였다. 증가된 activin A의 출처 확인을 위해 면역조직화학법을 시행하였다. 분석 결과 정상대조군의 척수에서는 혈관과 뉴런에서 activin A가 발현됨을 확인하였고 손상 후 염증 세포, 신경세포 와 몇몇 신경교세포에서 발현됨을 확인하였다. 이들은 면역형광염색법 결과 대식세포 와 별아교세포 임을 확인할 수 있었다. 결과적으로 SCI 후 activin A는 척수의 다양한 세포에서 일시적으로 증가하여 면역조절과 신경보호에 관여할 것으로 추측된다.

주요어 : activin A, spinal cord injury, 대식세포, 별아교세포

감사의 글

논문을 마치고 감사의 글을 시작하면서 지난 대학원생활을 떠올려보니 국방의 의무와 학업 두 마리 토끼를 놓치지 않기 위해 노력했던 제 모습과 그 모습에 따끔한 조언과 지지 그리고 격려를 아끼지 않으셨던 교수님과 선배님들에 대한 감사의 마음으로 2012년 새로운 한 해를 시작하고 있습니다.

학부생 시절부터 석사학위 과정까지 저의 부족함을 일깨워 주시고 항상 아낌없는 가르침을 주신 신태균 교수님께 깊은 존경과 감사의 말씀을 전합니다. 또한 본 논문이 만들어 지기까지 성심껏 심사를 해 주신 심기범 교수님, 김황룡 선배님께 감사의 말씀을 전합니다.

수의학이란 학문과 생명의 소중함을 가르쳐 주신 수의학과 김희석 교수님, 박전홍 교수님, 배종희 교수님, 이두식 교수님, 이경갑 교수님, 임윤규 교수님, 우호춘 교수님, 이영재 교수님, 정종태 교수님, 손원근 교수님, 김재훈 교수님, 황규계 교수님, 주홍구 교수님, 윤영민 교수님, 이주명 교수님, 강태영 교수님, 지영흔 교수님, 박현정 교수님, 한창훈 교수님, 그리고 고 이국경 교수님께 감사의 말씀을 전합니다.

아울러 배움의 길에 매진할 수 있게 많은 도움을 주신 제주특별자치도 동물위생시험소의 많은 분들께 감사의 말씀을 드리고 싶습니다. 김병학 소장님, 김명원 소장님, 허창현 소장님, 조순여 과장님, 김익천 과장님, 김장생 과장님, 최성두 계장님, 김창완 계장님, 김은주 계장님, 문성환 계장님, 김주아 계장님,

문혁 계장님, 김병수 계장님, 축산물분석계의 종철이형, 성훈이형, 승보, 성근이형, 혜진누나, 수현누나, 나래누나, 현주, 신혜, 검역계의 상우형, 김판석 주무관님, 준철형, 수경이모, 방역관리계의 경옥누나, 진영누나, 용준이형, 정헌이형, 유기동물보호센터의 철진이형, 동우형, 규석이, 공중방역수의사 1기 선배 으뜸이형, 2기 선배 경용이형, 형준이형, 3기 동기 영곤이형, 상훈이, 4기 정식이형, 5기 수교, 휘영이, 항상 나를 뒷받침 해 주었던 성환이까지 모두 깊은 감사의 말씀을 드립니다.

실험실 생활에서는 힘들 때 의지되어 주시고 도움과 격려를 아끼지 않으신 이용덕 박사님, 김철 선배님, 김승준 교수님, 강재윤 성배님, 진재광 박사님, 문창종 교수님, 강종철 선배님, 허승담 선배님, 박준수 선배님, 이기현 선배님, 안미정 박사님, 김희철 박사님, 이광협 선배님, 정경숙 선배님, 이지영 선배님, 김미아 선배님, 이태기 선배님, 변지성 선배님, 항상 옆에서 도와주신 정찬우 선배님 진심으로 감사 드립니다. 지금 일본에서 학업정진에 힘 쓰고있을 졸업동기 정태형, 부족한 선배지만 열심히 도와준 후배 지훈, 주연, 준영이형, 석원이형, 준우형, 성영이형, 원호, 원준, 한슬, 소희, 연지, 철호, 창남, 민수, 윤형, 은비, 그리고 많은 도움을 주신 김진희 선생님께 고마운 마음을 전합니다.

마지막으로 지금의 저를 있게 해 주시고 언제나 후원하고 아껴주시는 사랑하는 아버지, 어머니, 항상 형을 걱정해 주는 동생 진용에게 감사의 말을 전하고 싶습니다.